USDA

United States Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

4700 River Road
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20737

Richard Jupe, VP Product Design & Maintenance
Altria Client Services LLC
Center for Research and Technology
601 East Jackson Street
Richmond, Virginia 23219

Re: Confirmation of the regulatory status of tobacco varieties developed using CRISPR technology.

Dear Mr. Richard Jupe,

Thank you for your letter dated April 5, 2019, inquiring whether the tobacco (*Nicotiana tabacum*) varieties described in your letter are regulated articles under 7 CFR part 340. Your letter describes how *in vitro* derived mRNA transcripts and crRNA oligos designed to edit the genes of interest, were delivered by polyethylene glycol mediated transfection to tobacco protoplasts for the purpose of editing your genes of interest, resulting in the desired phenotype. The genes of interest and the trait are claimed as Confidential Business Information (CBI) in your letter of inquiry.

The Plant Protection Act (PPA) of 2000 gives USDA the authority to oversee the detection, control, eradication, suppression, prevention, or retardation of the spread of plant pests or noxious weeds to protect the agriculture, environment, and economy of the United States.

USDA regulates the importation, interstate movement and environmental release (field testing) of certain genetically engineered (GE) organisms that are, or have the potential to be, plant pests. Regulations for GE organisms that are, or have the potential to be, plant pests, under the PPA, are codified at 7 CFR part 340, "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There Is Reason To Believe Are Plant Pests." Under the provisions of these regulations, a GE organism is deemed a regulated article if it has been genetically engineered using a donor organism, recipient organism, or vector or vector agent that is listed in §340.2 and meets the definition of a plant pest; or that is an unclassified organism and/or an organism whose classification is unknown, or if the Administrator determines that the GE organism is a plant pest or has reason to believe it is a plant pest.

In your April 5, 2019 letter, you describe your tobacco protoplast transfection method to generate desired genome edits in tobacco genes. No constructs were introduced into the tobacco protoplasts; instead, *in vitro*-derived mRNA transcripts and gene specific crRNA oligos, designed to edit the genes of interest, were delivered by polyethylene glycol mediated transfection. The gene editing resulted in genetic changes consisting of deletions in the genes of interest, and the desired phenotype in the final product. The transfected protoplasts were grown using standard tissue culture methods and the plants were screened for nucleotide deletions in the target region of each gene, by PCR using gene-

specific primers and Miseq sequencing. To ensure that no foreign DNA was inserted into the tobacco genome in the edited plants, the mRNA used in transfections was treated with DNase to remove all template DNA prior to transfection. Additionally, to confirm that the mRNA used in transfection is not present in the genome of the edited plants, PCR primers were used to screen for the T7 promoter used to initiate mRNA *in vitro* transcription, and for two separate regions of the coding sequences necessary to edit the genes of interest. The presence of foreign nucleic acids was not observed in the modified plants.

Based on the information provided in your April 5, 2019 letter, the USDA has concluded that the described genome edited tobacco varieties are not regulated articles. These genome edited tobacco varieties do not contain DNA sequences derived from plant pests, were not modified using plant pests and no DNA sequences were introduced into the tobacco genome while using your tobacco protoplast transfection method as described in your letter.

Therefore, consistent with previous responses to similar letters of inquiry, USDA does not consider your gene edited tobacco varieties as described in your April 5, 2019 letter to be regulated pursuant to 7 CFR part 340. Additionally, tobacco is not listed as a Federal noxious weed pursuant to 7 CFR part 360, and USDA has no reason to believe that the phenotype resulting from the described modification of these genome edited tobacco varieties would increase the weediness of tobacco or of its sexually compatible relatives.

Please be advised that the importation of your genome edited tobacco seeds or plants, like all other tobacco, will be subject to applicable Plant Protection and Quarantine (PPQ), permit and/or quarantine requirements. For further information, should you plan to import these gene edited tobacco seeds or plants, you may contact the PPQ general number for such inquiries at (877) 770-5990.

Please be advised that your gene edited tobacco, while not regulated by APHIS under 7 CFR part 340 may still be subject to other regulatory authorities such as FDA or EPA. To inquire about the regulatory status of your product with the EPA, please contact Alan Reynolds at 703-605-0515. To inquire about the regulatory status of your product with the FDA, please contact Robert Merker at 240-402-1226.

Should you become aware at any time of any issues that may affect the Agency's conclusion regarding this inquiry, you must immediately notify the Agency in writing of the nature of the issue. We hope that you appreciate our commitment to plant health and support for the responsible stewardship for the introduction of GE plants.

Sincerely,

M. J. Firko

7/31/2019

Michael J. Firko, Ph.D.
APHIS Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date